

Evaluation of Heavy Metal Content in Main Stream Smoked Cigarettes and Non-smoked Tobacco in Kenya

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Abstract:

Background: Addictive cigarette smoking and the subsequent flux of different cigarette toxins including heavy metals have been implicated in the etiology of many smoking-related diseases such as cancer, stroke, rheumatoid arthritis and coronary heart disease. With over 5,000 chemicals in it, trace metals in cigarettes may have negative health impacts on humans.

Materials and Methods: The heavy metal content of the filler tobacco of the cigarettes and non-smoked tobacco, were quantified by atomic absorption spectroscopy. Pearson bivariate correlation analysis was applied to assess whether there are any associations between each pair of metals, regardless of the manufacturer/source.

Results: Filler tobacco of the cigarettes had Cd, Zn and Pb concentrations in the ranges of 0.091– 0.114, 0.157 – 0.310, and 7.145 – 11.873 µg/g, respectively. Chromium was not detected in any of the cigarette samples. Pearson bivariate correlation analysis indicated that there were no statistically significant correlations ($p < 0.05$) between the trace metals in the different cigarettes.

Conclusion: The trace metals in the cigarettes may have carcinogenic and non-carcinogenic health effects associated with direct or indirect inhalation of cigarette smoke. More and continuous monitoring and regulation of the ingredients of imported and locally produced tobacco products is recommended. A further study should assess the human health effects that may arise from smoking the cigarette brands.

Key Word: Heavy metals, tobacco, lead, cadmium, chromium

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I. Introduction

Smoking of cigarette tobacco has been implicated in the etiology of many life-threatening human diseases such as cancer, stroke, rheumatoid arthritis and coronary heart diseases [1, 2]. According to the World Health Organization (WHO), tobacco use accounts for at least 5 million deaths recorded annually [3, 4]. Further, it is estimated that tobacco use will account for not less than 8 million deaths annually by 2030, 80% of which will be registered in developing nations [3, 5]. Roughly, a life is lost every 10 seconds due to smoking of tobacco-based cigarettes. The frequent smoking of tobacco is appraised to be stimulated by nicotine which makes 0.6-3.0% (w/w) of its chemical composition [6]. This noxious stimulant is known to make smoking of cigarettes addictive [7]. The effects of nicotine are known to be analogous to those of illegal products from opium (*Papaver somniferum*) and cocaine (*Erythroxylum novogranatense* and *E. coca*) [8].

Addictive smoking of tobacco leads to continuous inhalation of more than 5,000 toxic and carcinogenic chemicals in cigarette smoke such as trace metals, polycyclic aromatic hydrocarbons, volatile organic compounds and nitrosamines [9, 10]. Among these toxicants, few studies have been undertaken on the role played by trace metals in the etiology of smoking-related diseases. The commonly used tobacco plant (*Nicotiana tabacum* L.) is now known to phytoaccumulate trace metals, particularly cadmium in its aerial parts (leaves) [5, 11]. The most commonly encountered trace metals in cigarettes with detrimental human health effects include lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni) and arsenic (As) [12]. The International Agency for Research on Cancer (IARC) reported that As, Ni, Cr(VI) and Cd compounds are human carcinogens while Pb is a Class 2B carcinogen that is additionally toxic to humans [13]. Further, trace metals such as cadmium and arsenic are also known to possess non-carcinogenic human health effects [14].

Against this background, there is a continuous need to conduct studies on the trace metal content of cigarettes and other tobacco-based products [12]. Only one study [15] previously reported on the Cd, copper, Zn, Cr and Pb content of two commercial cigarette brands (SM1, ES1) and one traditional cigarette (Trd) commercialized in Kenya. In the current study, we determined the levels of Cd, Cr, Zn and Pb in five commercialized cigarette brands (C1-C5) and a traditional cigarette (TDC) commonly smoked in Kenya.

II. Material And Methods

Sample collection

Unopened cigarette packs of five commercially available brands of imported and locally manufactured cigarettes (for confidential purposes were coded C1-C5) and a traditional cigarette (TDC) in Kenya were selected for this study. The samples were purchased in triplicate at various retail outlets in Moi University propinquity (Eldoret) and Mombasa, respectively on different dates and locations to account for potential variation in manufacturing dates and batches [16].

The components of cigarette, tobacco, filter and wrapping were separated. The mean weight of each cigarette was determined by weighing 5 sticks of each brand thrice using a precalibrated Mettler PM200 digital analytical balance (Marshall Scientific, Hampton, NH, USA) and averaging the results in each case [16, 17]. Further, the cigarettes were categorized according to their nicotine levels as light or normal according to the manufacturers' information [16].

Sample preparation

Composites of each brand were then prepared by removing the papers and filters from 60 cigarettes of 3 packs. The samples were subsequently dried in an oven at 80 °C for 6 hours and allowed to cool in a desiccator. The dried samples were separately ground in a mortar with a pestle until powdered finely as much as possible to pass through a 20-mesh sieve and to facilitate digestion.

The nitric acid wet-digestion method for plant tissues proposed by Campbell and Plank [18] was used in the current study due to its simplicity and speed. It is analogous to most plant tissue digestion methods, though it does not require the digest to reach a clear colourless endpoint prior to spectrometric quantification. Briefly, weighed aliquots (0.50 ± 0.10 g) of dry cigarette tobacco powders were transferred into 100 mL flat-bottomed flasks followed by addition of 5.0 mL of concentrated nitric acid. The flasks were covered with a watch glass and allowed to stand overnight. Thereafter, the flasks were heated at 180 °C for 30 minutes.

After, the digests were removed and allowed to cool and 2 mL of 30% hydrogen peroxide was added and digested at the same temperature and time in the same way to ensure complete digestion of the samples. The digested samples were then allowed to dry to 1 to 2 mL at 150 °C. After, 5 mL of 1% nitric acid was added to digest the residue which were then filtered through Whatman No. 1 filter paper into a 25 mL volumetric flask. This was topped up to the mark with deionized water. The filtrates were used for heavy metal analysis.

Trace metal quantification

All the samples were analyzed for Cd, Cr, Zn and Pb using AA 6300 Shimadzu double beam atomic absorption spectrophotometer (Shimadzu Corporation, Japan). Analyses, including quintuplicate sets of standards for each metal were run and the absorbances were used to compute the concentrations of the metals from the standard curves [19]. The results from the instrument were converted to the standard unit ($\mu\text{g/g}$ dry weight) to ease comparison with set compliance guidelines [20, 21].

Analytical quality control and quality assurance

All the analytical reagents employed in this investigation were of high analytical purity. Concentrated nitric and hydrochloric acids, hydrogen peroxide and standard salts of the trace metals were sourced from Merck (Darmstadt, Germany). The volumetric glassware used were pre-soaked in 5% nitric acid overnight, rinsed thrice with deionized water and then dried in an oven prior to the analyses. Standard solutions of Cd, Cr, Zn and Pb were prepared by dilution of the stock solutions ($1000 \mu\text{g/mL}$). The standard solutions were used for calibration and quality assurance for each of the analytical batches. Quality control was done with spiked samples analyzed once for every 1 in 10 samples. Recovery percentages from the spiked samples were from 96.7% to 101.5%. Method detection limits with reagent blanks were calculated and found to be $0.001 \mu\text{g/g}$ for all the metals. Analytical blanks were ran and subtractions were employed to correct the trace metal concentrations obtained. All samples were analyzed at least in triplicate.

Statistical analysis of data

All quantitative analytical data were captured in Microsoft Excel 365 (Microsoft Corporation, USA) for preliminary analysis and later exported into Minitab statistical software (Release 20, Minitab Inc., USA). Data were checked for normality prior to further statistical evaluation using the Kolmogorov-Smirnov test and

subsequently presented as means of triplicates with errors represented by standard deviations attached. Significant differences between mean concentrations of the investigated heavy metals were established using one-way ANOVA and separated using Turkey pairwise test. Bivariate Pearson correlation analysis was performed to establish if there existed any associations between the concentrations of each pair of the trace metals studied, irrespective of the source (manufacturer) [22]. Statistical analyses were performed at $p < 0.05$.

III. Result

Manufacturers' information and classification of the cigarettes

Preliminary assessments of weighed cigarette brands and their classification based on manufacturer information are shown in **Table 1**. According to conventional guidelines, cigarettes with nicotine levels ≤ 0.6 mg are considered light. Otherwise, the cigarettes are classified as normal. The cigarette brands used in this study were therefore classified as light or normal.

Table 1. Weights and classification of cigarette brands consumed in Kenya used in the study

Cigarette brand	Mean weight (mg)	Classification
C1	32.20 \pm 0.10	Light
C2	40.60 \pm 0.20	Light
C3	44.50 \pm 0.20	Light
C4	28.50 \pm 0.10	Normal
C5	24.90 \pm 0.20	Normal
TDC	Not applicable	Normal

The trace metal content of the cigarette brands sampled in this study are given in Table 2. Except for chromium, all the samples had detectable levels of the trace metals with zinc recording the highest concentration of 11.873 \pm 0.011 $\mu\text{g/g}$ in the normal brand (C4).

Table 2. Heavy metal content ($\mu\text{g/g}$ dry weight) of cigarette brands commercialized in Kenya

Brand	Cd	Cr	Pb	Zn
C1	0.091 \pm 0.003	BDL	0.310 \pm 0.010	8.581 \pm 0.001
C2	0.108 \pm 0.014	BDL	0.245 \pm 0.022	10.652 \pm 0.014
C3	0.114 \pm 0.011	BDL	0.237 \pm 0.110	7.145 \pm 0.003
C4	0.110 \pm 0.002	BDL	0.157 \pm 0.007	11.873 \pm 0.011
C5	0.090 \pm 0.017	BDL	0.220 \pm 0.016	10.402 \pm 0.009
TDC	0.150 \pm 0.001	BDL	0.265 \pm 0.005	5.717 \pm 0.016

BDL: Below method detection limit of 0.001 $\mu\text{g/g}$ dry weight

There were some differences in metal concentrations of cigarette brands produced by different manufacturers, suggesting differences in the source of tobaccos used by different companies. Investigation of potentially toxic trace metals in cigarette tobacco is important both from perspective of health studies connected with smoking and aspects of the uptake of trace elements by plants. We also compared the results of the trace metal contents obtained in our study with those reported by preceding studies in other parts of the world (**Table 3**).

Table 3. Heavy metal concentrations ($\mu\text{g/g}$) in various cigarette brands consumed in Kenya as compared to previous reports by preceding authors.

Country	Cd	Cr	Pb	Zn	Year	Author(s)
Finland	1.700	NR	2.400	50.000	1986	[23]
Canada	2.010	NR	NR	NR	1987	[24]
Austria	2.320	NR	NR	NR	1988	[25]
Italy	0.580	NR	7.390	NR	1989	[26]
Germany	1.950	1.110	1.200	49.800	1993	[27]
Hungary	1.890	NR	1.170	57.600	1995	[28]
Mexico	BDL	1.37	NR	24.000 – 65.000	1995	[29]
USA	0.905	NR	NR	35.100	1997	[30]

Country	Cd	Cr	Pb	Zn	Year	Author(s)
Egypt	NR	0.080 – 6.290	NR	76.800 – 190.000	1999	[31]
Turkey	1.700	1.630	1.020	NR	2001	[32]
India	0.900	5.000	4.300	39.500	2002	[33]
Jamaica	1.30	0.70 – 1.85	NR	33.000 – 84.000	2004	[34]
Nigeria	0.680 – 1.170	NR	NR	NR	2004	[35]
China	0.180	NR	0.64	NR	2005	[36]
Nigeria	0.700 – 2.300	NR	NR	NR	2005	[37]
Jordan	2.640	NR	2.670	55.620	2005	[11]
Pakistan	1.000 – 3.200	1.100 – 16.400	1.100 – 28.300	1.100 – 41.400	2006	[38]
Poland	0.610	NR	0.56	NR	2008	[39]
Pakistan	0.243 – 0.795	NR	2.266 – 24.080	2.500 – 27.330	2008	[40]
Mexico	0.180 – 3.070	0.150 – 0.490	NR	32.300 – 72.000	2008	[41]
Pakistan	1.660 – 2.960	NR	0.399 – 1.390	NR	2009	[42]
India	0.450	4.070	1.940	27.000	2010	[43]
Brazil	0.510 – 0.850	0.450 – 3.130	0.190 – 0.390	NR	2011	[22]
Spain	NR	NR	NR	385.000 – 947.000	2011	[44]
Iran	1.760 – 3.200	NR	1.050 – 3.100	18.100 – 42.200	2012	[17]
Ireland	1.730 – 2.020	NR	0.380 – 1.160	NR	2013	[45]
Singapore	0.019 – 0.034	0.042 – 0.066	0.125 – 0.164	1.700 – 5.453	2014	[46]
Spain	0.180	1.442	0.602	NR	2015	[47]
Kenya	0.063 – 0.093	1.590 – 3.605	6.637 – 7.066	0.219 – 1.143	2015	[15]
Egypt	1.600 – 5.900	NR	NR	NR	2017	[5]
Nigeria	5.900 – 7.940	18.260 – 34.940	17.210 – 74.780	47.020 – 167.310	2017	[16]
Iraq	BDL – 6.780	BDL – 6.730	1.240 – 9.260	0.170 – 3.110	2020	[10]
Kenya	0.091 – 0.114	BDL	0.157 – 0.310	7.145 – 1.873	2020	This study

NR: Not reported, BDL: Below method detection limit

Further, Pearson bivariate correlation analysis indicated that there was a positive correlation between Cd and Pb levels in the cigarettes whereas Cd and Zn, and Pb and Zn exhibited negative correlation with each other. However, these correlations were statistically insignificant at $p < 0.05$ (Table 4).

Table 4. Correlation among the heavy metals in cigarettes commercialized in Kenya.

Metal	Cd	Cr	Pb	Zn
Cd	-	-	0.009 (0.986)	-0.637 (0.174)
Cr		-	-	-
Pb			-	-0.614 (0.195)
Zn				-

Data are Pearson’s correlation coefficients and statistical significance (in parentheses).

Discussion of results

The concentration of trace metals in the cigarettes ranged from 0.091 to 0.15 µg/g for Cd, 0.16 to 0.31 µg/g for Pb, 5.72-11.87 µg/g for Zn while Cr was below the detection limit of 0.001 µg/g in all the samples (Table 2). Thus, the trace metal content of the cigarettes followed the chemical sequence: Zn > Pb > Cd > Cr.

The concentrations of Cd obtained were comparable to those reported previously for commercial cigarettes in Mexico [41], Kenya [15], China [36], Spain [47] and Iraq [10] (Table 3). Traditional non-smoked tobacco (TDC) showed higher levels of Cd than all the commercial cigarettes, corroborating a previous observation in our laboratory [15]. Cadmium is typically found at low concentrations in the environment and therefore less accumulated in plant tissues [48]. However, indiscriminate use of phosphate-based agrochemicals, thoughtless disposal of nickel-cadmium batteries into the environment, Cd metal incineration and production might accrue its environmental concentrations [49-51]. Ingestion of significant amounts of Cd causes renal and

hepatic dysregulations, hypertension, lung cancer and diminished reproductive potential in humans [52-55]. Further, Cd may get absorbed in the human digestive system and is capable of penetrating through the placenta during pregnancy, inducing DNA and membrane damages. Cadmium is also implicated in the etiology of calcium uptake inhibition and impairment of its subsequent retention in bones [56-58].

No chromium was detected in all the cigarette samples in the current study. This is in congruence with a 2020 report in Iraq by Haleem et al. [10] in which Cr was not detected in three commercial cigarette brands.

Lead on the other hand occurred in lower levels in comparison to most previous studies in other countries except for a Brazilian report [22] where the mean level of Pb obtained (0.27 µg/g) was comparable to those obtained in this study. The occurrence of Pb in environmental matrices have been grossly ascribed to the use of leaded petrol and inadvertent disposal of old lead batteries used in cars [57]. Lead is a toxin, a non-essential trace metal and the levels encountered in this study is deleterious to human health if inhaled in smoke over a long period of time [59]. Moreover, it is a rival that outcompetes essential metals of analogous characteristics such as Zn and calcium. Besides, it has been criminated for renal failure and liver degradation in humans [60]. Lead also retards interactive, survival, growth, developmental and metabolic processes in addition to upregulating mucus synthesis, as well as inducing neurodevelopmental damage that causes decreased intelligence quotient and behavioural problems [61, 62]. It may also induce reproductive problems, premature birth or reduce foetal growth in expectant women [62].

In contrast, the commercial cigarette brands in this investigation recorded higher Zn concentrations than the traditional cigarette brand (TDC), which is in complete agreement with a previous report in Kenya [15]. Only some previous studies in Pakistan [38, 40] recorded Zn concentrations that were lower or comparable to the ones obtained in this study. The occurrence of Zn in environmental matrices is usually from Zn used in old galvanized plumbing materials, Zn compounds in form of zinc-based paints, zinc alloys, dry cells, old and rusty galvanized roofing iron sheets and varnishes that may end up in the environment [57, 63]. Zinc is an essential trace metal [64] required for normal functioning of the immune system, normal brain activity, foetal growth and development. However, immoderate intake may impair immune function and lower the levels of high-density lipoproteins [65, 66].

The results of the current study confirmed previous reports that tobacco plants may phytoaccumulate trace metals preferentially in the leaves [11, 24, 39, 67]. Taken together, the disparity in the concentrations of trace metals in the cigarettes and those reported previously in other countries could be attributed to the differences in tobacco cultivation procedures, types, bioavailable trace metals in soils where the plants were cultivated as well as other soil characteristics [68, 69]. It is known that tobacco is a nutrient demanding but disease-prone plant which usually require agricultural inputs such as phosphorous-based fertilizers and pesticides to boost yield and protect its leaves from being destroyed by insect pests. Fertilizers and pesticides are known to contain trace metals which can easily be phytoaccumulated in herbaceous plants such as tobacco [48].

Further, we noted that there were non-significant correlations between Cd, Pb and Zn in the cigarette brands. In a similar correlative study, Viana et al. [22] reported the existence of excellent correlations between the concentrations of Ni and Pb, Cr and As, Ni and As, as well as Ni and Cr in some commercial cigarettes traded in Brazil.

IV. Conclusion

The current study has confirmed the occurrence of priority trace metals (Cd, Pb and Zn) in some cigarettes commercialized in Kenya. These may pose deleterious health effects to both direct and indirect smokers. More and continuous monitoring and regulation of the ingredients of imported and locally produced tobacco products is recommended. A further study should assess the human health effects that may arise from smoking the cigarette brands.

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